Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA

Temperature and Leaf Wetness Effects on Infection of Sugarcane by *Puccinia melanocephala*

Wilmer Barrera[1], Jeffrey Hoy[1] and Bin Li[2]

Authors' address: [1]Department of Plant Pathology and Crop Physiology; [2]Department of Experimental Statistics, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA (correspondence to J. Hoy; E-mail: jhoy@agcenter.lsu.edu)

Abstract

Brown rust epidemics in sugarcane, caused by *Puccinia melanocephala*, vary in severity between seasons. To improve the understanding of disease epidemiology, the effects of leaf wetness, temperature and their interaction on infection of sugarcane by the pathogen were studied under controlled conditions. Disease severity was low at 15 and 31°C regardless of leaf wetness duration. No infection occurred with a 4-h leaf wetness period. Increasing leaf wetness duration from 7 to 13 h lowered the temperature required for disease onset from 21 to 17°C. More infection occurred with 13 compared to 10 h of leaf wetness at 17°C, and severity decreased for all leaf wetness periods at 29 compared to 27°C. Postinfection suboptimal low and high temperatures increased the time required for lesion development and high temperatures decreased maximum disease severity. The observed effects of leaf wetness and temperature on infection by *P. melanocephala* could help explain the initiation, rate of increase and decline of brown rust epidemics in the field.

Introduction

Brown rust, caused by *Puccinia melanocephala* Syd. & P. Syd., can cause severe yield losses in susceptible sugarcane (*Saccharum* inter-specific hybrids) cultivars in most production regions (Raid and Comstock 2000). Yield reductions of 10–40% due to brown rust have been reported for susceptible cultivars, and severe epidemics can cause losses up to 50% (Purdy et al. 1983; Comstock et al. 1992; Raid and Comstock 2006). Losses as great as 22% were reported in Louisiana (Hoy and Hollier 2009). Several variables are associated with disease severity, including host resistance and pathogen virulence (Raid and Comstock 2000; Asnaghi et al. 2001; Shine et al. 2005), plant age (Comstock and Ferreira 1986), weather conditions (Sandoval et al. 1983; Irey 1987; Raid and Comstock 2006), and plant nutrition and soil characteristics

(Anderson and Dean 1986; Anderson et al. 1990; Johnson et al. 2007). Leaf wetness and temperature have been suggested to be the most important environmental variables affecting brown rust development (Purdy et al. 1983; Sandoval et al. 1983; Raid and Comstock 2000, 2006).

Leaf wetness defined as free moisture on the leaf surface for at least 8 h was found to be a requirement for brown rust infection (Raid and Comstock 2000). Infection was observed to be greatest when plants were incubated with leaf wetness for 14 h in the dark following inoculation (Ryan and Egan 1989). Water originating from dew has been suggested to be the most important moisture source during epidemics (Raid and Comstock 2006). Rain did not correlate with rust severity in one study (Comstock and Ferreira 1986), and although it creates leaf wetness, rain also could have a detrimental effect by washing urediniospores from the leaf surface (Raid and Comstock 2006).

Urediniospore germination and appressorium formation occurred over a wide temperature range from 5 to 34°C, with an optimal range from 15 to 30°C (Sotomayor et al. 1983). In other studies (Sahni and Chona 1965; Hsich et al. 1977), optimal germination occurred between 21 and 26°C, with urediniospore germination rapidly decreasing with temperatures exceeding 30°C (Sotomayor et al. 1983). Urediniospores were reported to rapidly lose viability when temperatures increase above 35°C (Purdy et al. 1983).

The effects of environmental variables on postinfection development of brown rust have not been studied. However, temperature has been shown to affect latent period length and infection efficiency for other rust fungi (Eversmeyer et al. 1980; Webb and Nutter 1997).

High temperatures under field conditions have been linked with lower brown rust severity and the decline of epidemics (Liu and Bernard 1979), but the effects of temperature on infection are not well understood. It

has been suggested that high temperature affects the viability of spores (Purdy et al. 1983) and their germination (Sotomayor et al. 1983). A significant reduction in the number of trapped urediniospores in spore samplers followed the occurrence of maximum daily temperatures above 30°C in Florida (Irey 1987). Additionally, the natural decline of epidemics was observed when maximum daily temperatures exceeded 32°C in Louisiana (Barrera 2010).

Information is incomplete concerning the effects of leaf wetness and temperature and their interaction on leaf infection by *P. melanocephala* and disease development. The objectives of this study were to investigate under controlled conditions the effects of leaf wetness and temperature on infection by *P. melanocephala* and disease severity, including the postinfection effect of temperature, in order to gain a better understanding of how the interaction of these variables affects brown rust epidemic development.

Materials and Methods

Urediniospores of *P. melanocephala* were collected with a vacuum device (Model DC515; DEWALT Industrial Tool Corp., Baltimore, MD, USA) equipped with a cyclone spore harvester (G-R Manufacturing, Manhattan, KS, USA) from naturally infected leaves of multiple susceptible cultivars during 2009 and 2010. Spores were tested for viability by plating on water agar overnight at room temperature ca. 23°C and preserved at −80°C. These spores were used to inoculate leaves on sugarcane plants that were then exposed to different combinations of temperature and hours of leaf wetness. Urediniospore viability was determined by plating on water agar at room temperature at the time of each inoculation and ranged from 22 to 30% during the experiments.

Plants of brown rust susceptible cultivar Ho 95-988 (Tew et al. 2005) produced from single-bud cuttings in the greenhouse were used in the experiments. Plants were between 60 and 85 days old with approximately four fully emerged leaves at the time of inoculation. The substrate used for growing the plants was a 1 : 1 mixture of silt loam soil and sand. Plants were fertilized with 15-11-15 N-P-K 2 weeks prior to inoculation with the pathogen.

Plants were inoculated with a urediniospore suspension containing approximately 1×10^6 spores/ml. To achieve this, spores were suspended in a solution containing distilled water and 0.1% Tween 20. Spore concentration was assessed with a haemocytometer and adjusted accordingly. Inoculum was applied to both sides of one fully emerged leaf per plant with a brush until a film of moisture was visible. To maintain defined periods of leaf wetness, the inoculated leaf was introduced in a horizontally positioned glass test tube (70 ml, 25×200 mm) containing 3 ml of distilled water. Tubes were sealed with Parafilm (Pechiney Packaging Company, Chicago, IL, USA) and then held in a stable horizontal position with tube racks. Entire plants including inoculation chambers were placed inside an upright cabinet incubator (Model 3740; Forma Scientific, Marietta, OH, USA) at a given temperature in the dark. During the infection period, temperature was monitored with a thermocouple temperature sensor (Model 3667s; Spectrum Technologies, Plainfield, IL, USA). The temperature variation within the incubator never exceeded ±1°C. After the leaf wetness period for a given treatment was completed, the glass tube was removed from the leaf, and the leaf was allowed to air dry. After inoculation, plants were placed on shelves with 12 h/day artificial lighting for 14 days at room temperature (23 ± 1°C). Disease severity was assessed after 14 days by counting at 10× magnification the total number of necrotic lesions for each inoculated leaf. Lesion density per cm^2 was calculated by dividing the total number of lesions by the leaf area. Leaf area was determined by image analysis with Assess software (APS Press, American Phytopathological Society, St. Paul, MN, USA).

Temperatures tested were 15, 17, 19, 21, 23, 25, 27, 29 and 31°C, and leaf wetness periods tested were 4, 7, 10 and 13 h. All temperatures were evaluated in combination with each of the leaf wetness periods. Every combination consisted of four replicates that received the same leaf wetness treatment within a single incubator at each temperature. The experiment was performed twice.

The effect of temperature, leaf wetness and their combined effects on infection was analysed with the Randomization test (R statistical program; R Foundation for Statistical Computing, Vienna, Austria). Due to lack of normality in the experimental data, statistical analyses of the different levels of temperature and leaf wetness were made with Friedman's nonparametric test (InfoStat Statistical Software, National University of Córdoba, Argentina).

The effects of temperature on the time required for lesion development and maximum disease severity were studied using the same plant growth and inoculation procedures as described previously. To insure leaf wetness and temperature would be favourable for infection, inoculated leaves were exposed to an 18-h leaf wetness period at 23 ± 1°C. Then, the glass tube was removed from inoculated leaves, and plants were placed inside incubators (Model 3740; Forma Scientific) under a 12-h day at a given temperature for 14 days. Temperatures tested were 15, 20, 25, 30 and 32°C. Plants were temporarily removed from the incubator, and lesion development was quantified following 4, 6, 8, 10, 12 and 14 days of incubation. Severity over time was assessed as described previously. Time required for 50% lesion development and the maximum severity were determined for each treatment. Lesions occurring in high numbers began to coalesce over time in some treatments, so maximum severity was compared among treatments at day 10. Every treatment consisted of six plants with a single inoculated leaf per plant placed together in an incubator at each temperature. The experiment was performed twice. Statistical analysis of the data was conducted

with the PROC GLM procedure, and treatment means were compared with Tukey's test (SAS Institute, Cary, NC, USA).

Results

Effects of temperature and leaf wetness on infection

Leaf wetness duration and temperature each affected infection by *P. melanocephala* in both experiments ($P < 0.001$), and the combined effects also were highly significant ($P < 0.001$). Differences were detected among wetness periods and temperatures (Fig. 1). Leaves exposed to 4 h of leaf wetness did not develop symptoms regardless of the incubation temperature following inoculation, and no infection occurred with a 7-h wetness period at 15 and 31°C. A few lesions developed with a 7-h wetness period at 17 and 19°C. Increasing the length of the leaf wetness period from 7 to 10 or 13 h resulted in a progressively higher number of lesions at 17 and 19°C. Increasing leaf wetness from 7 to 13 h resulted in an increase in severity at temperatures ranging from 17 to 27°C. Severity was higher in Experiment 2 for leaf wetness periods of 7 and 10 h at disease favourable temperatures of 23–29 and 21–29°C, respectively.

Inoculation resulted in successful leaf infection for the entire temperature range of 15–31°C, but differences were detected among temperature treatments (Fig. 1). Disease severity was low at 15 and 31°C regardless of leaf wetness period. Temperature had a differential effect on lesion number depending on the length of the leaf wetness period. An optimal temperature range (no differences among treatment severity values) of 21–27°C was evident with a 7-h wetness period, but an optimal temperature range of 17–27°C was detected with a 13-h wetness period. Appreciable infection occurred at 29°C only in Experiment 2.

Effect of temperature on postinfection brown rust development

Postinfection temperature affected the time required for lesion development and maximum severity (Table 1). The time required for 50% lesion development was 10 days at 15°C. Less time was required at 20 and 25°C. Seven and 8 days were required at 20°C in Experiments 1 and 2, respectively, and 7 days were required at 25°C. The times required for 50% lesion development at 30 and 32°C were similar to the time required at 15°C. Ten and 11 days were required at 30 and 32°C, respectively, in Experiment 1. In Experiment 2, the 9 days required at 30°C was not different than the 8 days required at 20°C, while more time (12 days) was required at 32°C. Coalescing lesions made it difficult to accurately determine the maximum disease severity resulting from infection; however, a few differences were detected among postinfection temperatures (Table 1). The highest severity occurred at 15 and 20°C in Experiment 1 and 15, 20 and 25°C in Experiment 2. Lesion numbers were higher at 15, 25 and 30°C in Experiment 2.

Discussion

Infection by *P. melanocephala* assessed as disease severity was affected by leaf wetness and temperature, and changes in one variable influenced the effect of the other. Successful infection occurred following a 7-h but not 4-h leaf wetness period. Five- and 6-h wetness periods were not compared, but a previous study (Raid and Comstock 2000) suggested an 8-h leaf wetness period was required for infection. Low levels of infection occurred at 15 and 31°C regardless of leaf wetness period. An optimal temperature range of 21–27°C was evident with a 7-h wetness period, but the optimal range expanded to 17–27°C when the leaf wetness period increased to 13 h. Increasing the length of the leaf wetness period from 7 to 10 or 13 h generally resulted in the development of higher
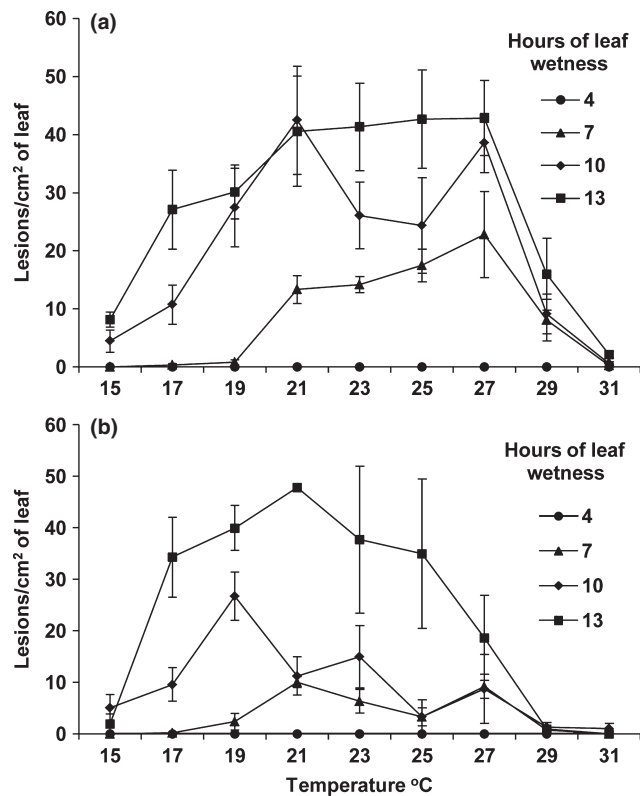

Fig. 1 Effect of temperature and leaf wetness duration on brown rust infection and severity. (a) Experiment 1; (b) Experiment 2. Bars on treatments indicate mean standard error

Table 1
Effect of postinfection temperature on the time course for brown rust lesion development and on maximum disease severity

| Variable | Experiment | Temperature (°C)[a] | | | | |
		15	20	25	30	32
Days until the formation of 50% of lesions	1	10 a	7 b	7 b	10 a	11 a
	2	10 ab	8 cd	7 d	9 bc	12 a
Maximum disease severity (lesions/cm²)	1	33 a	52 a	17 b	6 b	10 b
	2	61 a	51 ab	60 a	27 b	4 b

[a]Mean values within a row followed by a different letter were significantly different ($P \leq 0.05$) according to Tukey's test.

numbers of lesions within the optimum temperature range.

The reasons for higher disease severity in Experiment 2 at temperatures within the optimum range with 7 and 10 h of leaf wetness are uncertain. The urediniospore source was different for the two experiments, but spore viability was similar. Research on two other *Puccinia* species on wheat, *P. striiformis* and *P. triticina*, demonstrated that low light quantity prior to inoculation reduced infection efficiency (Vallavieille-Pope et al. 2002). In this study, Experiment 1 was conducted from December to February (winter), while Experiment 2 was conducted during July and August (summer), and solar radiation measurements from a nearby weather station for the plant growth period prior to and during the two experiments averaged 0.109 and 0.232 watts⁄m², respectively. This difference suggests that a similar phenomenon could exist for brown rust, but additional research is needed to confirm whether light quantity also can affect infection efficiency by *P. melanocephala*. The results from this study were consistent across experiments for the minimum and maximum leaf wetness and temperature conditions needed for infection. These are the parameters of greatest influence in the epidemiology of the disease.

The 7-h leaf wetness period needed for infection by *P. melanocephala* was similar to a previous study that suggested a requirement for at least 8 h of exposure to liquid water for infection (Raid and Comstock 2000). In addition, the formation of lesions at temperatures within an optimum range of 17–27°C coincides with *P. melanocephala* urediniospore germination results obtained in other studies. Optimal germination temperatures ranged from 21 to 26°C (Sahni and Chona 1965; Ryan and Egan 1989) and 15 to 30°C (Sotomayor et al. 1983).

Visible lesion formation was observed 6 days after inoculation in these experiments, but symptom development continued until the 14th day. Sotomayor et al. (1983) reported the rupture of the epidermis and formation of urediniospores beginning 7 days after inoculation. A time period of approximately 8–11 days was assumed to be necessary between spore germination and the production of a new generation of spores by Irey (1987).

Factors affecting postinfection lesion development have not been addressed previously for brown rust. The current research found that postinfection temperature affects the time required for lesion formation and maximum disease severity. Lesion development was more rapid at the moderate temperatures of 20 and 25°C. Similar results were found for infection by *P. recondita* in wheat (Eversmeyer et al. 1980). As with *P. melanocephala*, temperatures below and above the optimum range increased latent period length. However, increasing temperatures decreased latent period length for alfalfa rust caused by *Uromyces striatus* but did not affect infection efficiency (number of pustules per leaf) (Webb and Nutter 1997). Maximum brown rust severity was reduced by postinfection suboptimal high temperatures of 30 and 32°C but not by the suboptimal low temperature of 15°C.

Field application of the study results will require additional research and appropriate interpretation. A 7-h minimum requirement and higher infection with increasing leaf wetness period could explain disease development associated with dew formation under field conditions. Explaining disease initiation and severity under field conditions where temperature is constantly changing is more challenging than under controlled conditions. However, the results of this study are consistent with the results of previous field studies. In the Dominican Republic, ambient temperatures above 35°C were linked with reduced disease severity (Liu and Bernard 1979), and lower aerial concentrations of urediniospores followed maximum ambient temperatures above 30°C in Florida (Irey 1987). In Hawaii, maximum brown rust development was observed when mean minimum monthly temperatures were 20°C or below (Comstock and Ferreira 1986).

The minimum, optimum and maximum values determined in this study for leaf wetness and temperature and the results of their combined effects could be helpful when making comparisons with data from natural epidemics to improve our understanding of conditions required for epidemic onset, rate of increase over time and eventual decline of epidemics. This could eventually allow the development of a disease advisory or field-level forecasting system to predict the initiation and potential severity of brown rust epidemics in susceptible sugarcane cultivars during each growing season.

References

Anderson DL, Dean JL. (1986) Relationship of rust severity and plant nutrients in sugarcane. *Phytopathology* **76**:581–585.

Anderson DL, Raid RN, Irey MS, Henderson LJ. (1990) Association of sugarcane rust severity with soil factors in Florida. *Plant Dis* **74**:683–686.

Asnaghi C, D'Hont A, Glaszmann JC, Rott P. (2001) Resistance of sugarcane cultivar R 570 to *Puccinia melanocephala* isolates from different geographic locations. *Plant Dis* **85**:282–286.

Barrera WA. (2010) Effects of environmental variables and crop growth on development of brown rust epidemics in sugarcane. M.S. Thesis. Louisiana State University Agricultural Center, Baton Rouge, 89 pp. Online publication. ETD-11182010-115435.

Comstock JC, Ferreira SA. (1986) Sugarcane rust: factors affecting infection and symptom development. *Proc Int Soc Sugar Cane Technol* **19**:402–410.

Comstock JC, Shine JM, Raid RN. (1992) Effect of sugarcane rust on growth and biomass. *Plant Dis* **76**:175–177.

Eversmeyer MG, Kramer CL, Browder LE. (1980) Effect of temperature and host:parasite combination on the latent period of *Puccinia recondita* in seedling wheat plants. *Phytopathology* **70**:938–941.

Hoy JW, Hollier CA. (2009) Effect of brown rust on yield of sugarcane in Louisiana. *Plant Dis* **93**:1171–1174.

Hsich W, Lee C-S, Chan SL. (1977) Rust disease of sugarcane in Taiwan: the causal organism *Puccinia melanocephala* Sydow. *Taiwan Sugar* **24**:416–419.

Irey M. (1987) Effect of the environment on sugarcane rust epidemics in Florida. *J Amer Soc Sugar Cane Technol* **7**:30–35.

Johnson RM, Grisham MP, Richard EP Jr. (2007) Relationship between sugarcane rust severity and soil properties in Louisiana. *Phytopathology* **97**:748–755.

Liu LJ, Bernard F. (1979) Sugarcane rust in the Dominican Republic. *Sugarcane Pathol Newsl* **22**:5–7.

Purdy LH, Liu LJ, Dean JL. (1983) Sugarcane rust, a newly important disease. *Plant Dis* **67**:1292–1296.

Raid RN, Comstock JC. (2000) Common rust. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (eds) *A Guide to Sugarcane Diseases*. Montpellier, France, La Librairie du Cirad, pp 85–89.

Raid RN, Comstock JC. (2006) *Sugarcane Rust Disease*. Agronomy Department document SS-AGR-207. Gainesville, FL, USA, University of Florida⁄IFAS.

Ryan CC, Egan BT. (1989) Rust. In: Ricaud C, Egan BT, Gillaspie AG Jr, Hughes CG (eds) *Diseases of Sugarcane: Major Diseases*. Amsterdam, The Netherlands, Elsevier Science Publishers B.V., pp 189–210.

Sahni ML, Chona BL. (1965) Studies on sugarcane rust in India. *Indian Phytopathol* **18**:191–203.

Sandoval I, Picornell V, Chaves R, Ramos N. (1983) Puccinia melanocephala H&P. Syd.: Biological and ecological aspects. *Proc Int Soc Sugar Cane Technol* **18**:845–853.

Shine JM Jr, Comstock JC, Dean JL. (2005) Comparison of five isolates of sugarcane brown rust and differential reaction of six sugarcane clones. *Sugar Cane Int* **23**:24–29.

Sotomayor IA, Purdy LH, Trese AT. (1983) Infection of sugarcane leaves by *Puccinia melanocephala*. *Phytopathology* **73**:695–699.

Tew TL, Burner DM, Legendre BL, White WH, Grisham MP, Dufrene EO, Garrison DD, Veremis JC, Pan Y-B, Richard EP Jr. (2005) Registration of 'Ho 95-988' sugarcane. *Crop Sci* **45**:1660.

Vallavieille-Pope C, Huber L, Leconte M, Bethenod O. (2002) Preinoculation effects of light quantity on infection efficiency of *Puccinia striiformis* and *P. triticina* on wheat seedlings. *Phytopathology* **92**:1308–1314.

Webb DH, Nutter FW Jr. (1997) Effects of leaf wetness duration and temperature on infection efficiency, latent period, and rate of pustule appearance of rust in alfalfa. *Phytopathology* **87**:946–950.